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A bioassay for the toxicity of sediment to marine macrobenthos

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The final Revision of Ocean Dumping Regulations and Criteria published by the Environmental Protection Agency[1] (EPA) requires, under certain conditions, bioassays of the liquid, suspended particulate, and solid phases of dredged sediments proposed for disposal in the marine environment. Procedures for these bioassays were recently developed by a joint technical committee of EPA and the Corps of Engineers.[2] Results of experiments that contributed to the development of the bioassay for the solid or settleable phase of dredged material are presented in this report.

The objective was to develop a test for the acute toxicity of the settleable components. The bioassay was designed not to be sensitive to burial or changes in sediment particle size distribution. These effects certainly can disrupt benthic communities within dump site boundaries, but are unavoidable even during the disposal of unpolluted sediment. The aim was to measure the toxicity, if any, of dredged sediments whose disposal might result in the trophic or physical transport of pollutants beyond the dump site.

The bioassay technique roughly simulates the dumping of dredged material into the sea. Five macrobenthic invertebrates representing different taxonomic and trophic positions were selected as the test species because of the vulnerability of the benthos to the settleable portion of dredged sediments. These organisms were allowed to acclimate to control (nonpolluted) sediment and were then covered in different replicates by a layer of either test or control sediment. Except during the 1-hour period of initial settling, the bioassay aquaria were supplied with a continuous flow of sea water that carried away the dissolved and suspended phases. Survival relative to controls after 10 days of exposure was the primary response criterion.

Bioassays were conducted for sediments from the Atlantic Ocean, Gulf of Mexico, and Pacific coast of the U. S. The collection sites were chosen to provide a comparison of the toxicity of sediments that had been exposed to a variety of kinds and degrees of pollution.

MATERIALS AND METHODS

Sediment samples. Benthic bioassays were performed on sediments from Yaquina Bay, Coos Bay (4 stations) and the Skipanon River (Oreg.); the Duwamish River (3 stations), Elliott Bay, and Puget Sound (Wash.); Houston Ship Channel (Tex.), (5 stations); Bailey Creek (Va.); and the Raritan River (N. J.) (Table I). There are no significant sources of pollution in the vicinity of the Yaquina Bay collection site. The four Coos Bay stations are in a channel next to a number of log loading docks. The Skipanon River station is adjacent to a fishing marina. Bailey Creek is near the site of the Kepone contaminations of the James River. The Duwamish, Raritan, and Houston Channel systems are exposed to a variety of industrial and domestic sources of pollution. Slip No. 1 in the Duwamish was the site of a polychlorinated biphenyls (PCB) spill on September 13, 1974. The benthic bioassay sediment sample from slip No. 1 was collected after two attempts had been made to clean up the PCB contamination.[3] The Elliott Bay station is located at an experimental dump site for Duwamish River sediments.[4] Houston Channel Stations II, III, IV, and V are near a chlor-alkali plant, a pulp and paper mill, an oil refinery, and a wastewater outfall, respectively. Station I is off Morgan's Point at the entrance to the channel.

The sediment samples were maintained at 4°C from the time of collection until the initiation of the bioassay, a period that did not exceed 2 weeks. The samples were either sieved through a 0.5-mm screen or frozen to remove

TABLE I. Source and particle size distribution of sediment used in benthic bioassays.

Experiment Number	Sediment Type	Sediment Size Distribution			
		% Sand	% Silt	% Clay	Mean Grain Size ($\times 10^{-6}$ m)
1	Yaquina Bay Control	98.7	0.3	1.0	209
2	Yaquina Bay Control	98.6	0.5	0.9	204
	Fraction of Control:				
	Silt	3.7	93.6	2.7	26
	Very Fine Sand	87.0	9.8	3.2	69
	Fine Sand	100.0			188
	Medium Sand	100.0			272
	Coarse Quartz Sand	100.0			859
	Very Coarse Quartz Sand	100.0			1 395
	Control Fractions-Quartz Sand Composite	86.3	13.0	0.7	259
3	Yaquina Bay Control	98.9	0.3	0.8	202
	Duwamish River Turning Basin	24.2	64.3	11.5	21
	Duwamish River Slip No. 1	14.6	60.4	25.0	12
	Duwamish River Mouth	29.7	57.5	12.8	26
	Elliott Bay	39.0	51.0	10.0	21
	Puget Sound	11.4	45.2	43.7	7
4	Yaquina Bay Control	99.0	0.3	0.7	203
	Coos Bay Station I	98.7	0.4	0.9	289
	Coos Bay Station II	66.8	25.7	7.5	62
	Coos Bay Station III	94.3	3.3	2.4	193
	Coos Bay Station IV	96.0	2.1	1.9	203
5	Yaquina Bay Control	98.7	0.5	0.8	196
	Houston Ship Channel Station I	5.2	48.0	46.8	6
	Houston Ship Channel Station II	2.6	38.6	58.9	4
	Houston Ship Channel Station III	11.2	28.5	50.3	7
	Houston Ship Channel Station IV	31.0	58.7	20.3	19
	Houston Ship Channel Station V	40.3	38.1	21.6	29
6	Yaquina Bay Control	96.5	1.8	1.7	187
	Bailey Creek	41.9	31.5	26.5	29
7	Yaquina Bay Control	98.4	0.6	1.0	200
	Skipanon River	6.2	68.8	25.0	11
8	Yaquina Bay Control	99.1	0.2	0.7	212
	Raritan River	23.1	38.4	38.5	16

macrofaunal specimens. Sediment particle size distribution was determined for the sand fraction by use of a Wentworth screen series and for the silt-clay fraction by the pipette method.[5]

Test species. Test specimens were collected with a bottom dredge from Yaquina Bay and removed from the sediment by sieving through a 1.0-mm screen. The five species used in each experiment included the pelecypods, *Protothaca staminea* and *Macoma inquinata*, the polychaete, *Glycinde picta*, and the amphipod, *Paraphoxus epistomus*. The fifth species was one of the following cumaceans: *Diastylis alaskensis*, *Lamprops quadriplicata*, *Diastylopsis dawsoni*, or *Cyclaspis* sp., depending on availability in Yaquina Bay at the time of each experiment. These species represent different feeding types and taxa that often dominate continental shelf and estuarine benthic assemblages.

Because the test organisms occurred natur-

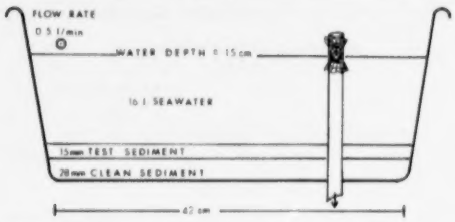

FIGURE 1. Diagram of the benthic bioassay aquarium.

ally at the Yaquina Bay sediment collection site, that sediment was used as a control against which the toxicity of sediments from other areas was compared.

Bioassay procedure. The bioassay aquaria were polyethylene boxes with an approximate capacity of 25 l and a bottom area of 1 200 cm^2 (Figure 1). They were supplied with a continuous flow of sea water (0.5 l/min) at ambient temperature and salinity (9.5 to 16.8°C, 23.5 to 32.7‰ during the experiment series).

Eight experiments were conducted from May 1976 until April 1977. The basic design was to place a 28-mm layer of control sediment on the bottom of each aquarium. Test organisms were randomly distributed so that each tank contained 20 individuals of each of the five species. They were allowed to "acclimate" for a 48-hour period during which time dead or lethargic specimens were replaced. Treatments were randomly assigned to the aquaria after the acclimation period. The test sediment was then introduced by distributing it evenly over the water surface and allowing it to settle for 1 hour while the sea water was turned off. After 1 hour the normal flow resumed and the dissolved and suspended fractions were flushed out. The solid or settleable fraction remained as an even layer on top of the control sediment. After 10 days, surviving individuals were recovered by sieving the sediment through a 0.7 mm screen, and counted.

Statistical analysis. Differences in mean survival between the control and test sediments were compared for the individual species and the entire assemblage. Dunnett's procedure [6] for comparing more than one mean with a control was used for Experiments 1, 3, 4, and 5. Student's t test was used for Experiments 6, 7, and 8, which involved only one experimental treatment. Because the objective was to determine if survival was less than in the control, one-tailed Student and Dunnett t tables were used to determine significant differences.

RESULTS

Effects of burial. The first experiment was designed to determine the effects of burial by Yaquina Bay sediment. Sediment layers 5-, 10-, 15-, 20-, 25-, and 30-mm thick were deposited on top of the 28-mm bottom sediment layer. A seventh treatment consisted of a nonburial control—that is, an overlying layer was not deposited. Replicate bioassays were conducted for each sediment depth and the nonburial control.

Mean survival in the nonburial control for each individual species and for the entire assemblage was not significantly different from survival at any of the burial depths (Table II). With one exception, the number of survivors in individual aquaria ranged from 89 to 97 specimens (x = 93.5). An exception, or

TABLE II. Mean survival of a macrobenthic assemblage 10 days after deposition of Yaquina Bay (control) sediment layers 0 to 30 mm in thickness. Each treatment was replicated twice.

		Mean Number Recovered						
		Non-burial Control	Sediment Layer (mm)					
Species	Initial Number	0	5	10	15	20	25	30
Protothaca staminea	20	20.0	20.0	19.5	20.0	18.0	19.5	19.0
Macoma inquinata	20	20.0	20.0	19.5	20.0	18.5	19.5	20.0
Glycinde picta	20	17.5	17.0	18.0	18.5	17.5	16.5	17.5
Paraphoxus epistomus	20	18.5	19.5	17.5	17.5	18.0	18.0	19.5
Cumacea	20	19.0	19.0	18.0	16.5	17.0	18.5	15.5
Total	100	95.0	95.5	92.5	92.5	89.0	92.0	91.5

TABLE III. Effects of changes in sediment particle size distribution on the survival of the macrobenthic assemblage. Mean grain size ($\times 10^{-6}$m) of the sediment types is given in parentheses.

Species	Initial Number	Number Recovered: Yaquina Silt (26)	Yaquina Very Fine Sand (69)	Yaquina Fine Sand (188)	Yaquina Medium Sand (272)	Quartz Coarse Sand (859)	Quartz Very Coarse Sand (1395)	Composite[a] (259)	Yaquina Control (204)
Protothaca staminea	20	20	20	20	20	20	20	20	20
Macoma inquinata	20	18	19	18	20	20	20	20	18
Glycinde picta	20	17	19	19	16	19	20	20	17
Paraphoxus epistomus	20	15	18	15	18	13	15	14	16
Cumacea	20	19	15	19	14	4	7	19	14
Total	100	89	91	91	88	76	82	93	85

[a] Composite of equal volume of all test sediments except the intact Yaquina control.

"outlier," (81 survivors) occurred in one of the 20-mm burial treatments. In the two replicates buried under 15 mm of sediment, 91 and 94 of the initial 100 specimens were recovered. This was considered to be a sufficiently low control mortality for routine toxicity bioassays. After the first experiment, only one sediment depth (15 mm) was deposited on top of the bottom layer containing the test organisms. Thus, the control replicates received 15 mm of Yaquina sediment on top of 28 mm of Yaquina sediment. The experimental replicates received 15 mm of test sediment on top of 28 mm of Yaquina sediment.

Effects of changes in sediment particle size. The second experiment examined the effects of different particle size distributions. The eight treatments were not replicated. They included Yaquina sediment fractions with mean grain sizes of 26, 69, 188, and 272 μm; coarse (859 μm) and very coarse (1 359 μm) quartz sands obtained from an aquarium supply company; a composite of the six preceding sediments (259 μm); and intact sediment (204 μm).

Obvious effects of particle size alterations were found only for the survival of cumaceans in the coarse and very coarse sand fractions (Table III). Mean recovery for the entire assemblage on the sediment fractions with mean grain sizes between 26 and 272 μm was 89.5. individuals. Within this group, the unsieved control sediment had the lowest recovery (85 individuals). The mean particle size of sediments used in later experiments ranged from 4 to 289 μm. Further evidence that fine grain sediments did not affect survival was obtained in the bioassay of Skipanon River sediment, which had an 11 μm mean grain size, but no significant difference from the control in the survival of any species or the entire assemblage.

Sediment toxicity bioassays. The last six experiments involved bioassays of sediment from various coastal regions of the U. S. All treatments in the first three of these toxicity bioassays were replicated twice. Sediments collected along a transect from the Turning Basin, Slip No. 1, and mouth of the Duwamish River and from adjacent Elliott Bay and Puget Sound were used in the third experiment. The fourth and fifth experiments included sediment from four stations in Coos Bay and five stations in the Houston Ship Channel, respectively. There were five replicates of the Yaquina control and the test sediment in the last three experiments. Test sediment from one station in Bailey Creek, Skipanon River, and Raritan River were used in the sixth, seventh, and eighth experiments, respectively.

There were no significant differences from the controls in the mean survival of any species exposed to sediment from the Skipanon River (Experiment 7) or any of the four Coos Bay stations (Experiment 4) (Table IV).

The differences between mean survival of total individuals in the controls and in sediment from the Raritan River (Experiment 8), Bailey Creek (Experiment 6), and the five stations in the Houston Ship Channel (Experiment 5) were all significant (Table IV). The impact of these sediments was most obvious in the survival of *Paraphoxus epistomus* and the Cumacea. The mean survival of *Glycinde picta* in the Houston Channel Stations III and V replicates and the mean for *Macoma inquinata* in the Bailey Creek and Duwamish Slip No. 1 sediments were also significantly different from the controls.

TABLE IV. Mean survival of the macrobenthic assemblage in sediment toxicity bioassays. Twenty individuals of each species were initially placed in each replicate.

Experiment Number	Sediment Type	Number of Replicates	Mean Survival					
			Total	*Protothaca staminea*	*Macoma inquinata*	*Glycinde picta*	*Paraphoxus epistomus*	Cumacea
3	Yaquina Control	2	87.5	19.5	19.5	14.0	19.5	15.0
	Duwamish Turning Basin	2	69.0[a]	20.0	17.0	17.5	4.0[a]	10.5
	Duwamish Slip No. 1	2	65.0[a]	20.0	13.0[a]	16.0	9.0[a]	7.0
	Duwamish Mouth	2	81.5	20.0	16.0	17.0	17.0	11.5
	Elliott Bay	2	71.0[a]	20.0	17.5	15.5	12.0[a]	6.0
	Puget Sound	2	76.0[a]	20.0	16.5	14.0	17.5	8.0
	Yaquina Control	2	94.0	20.0	18.5	19.5	19.0	17.0
	Coos Bay Station I	2	91.0	20.0	19.5	20.0	18.5	13.0
	Coos Bay Station II	2	92.0	20.0	19.5	18.0	18.5	16.0
	Coos Bay Station III	2	93.0	20.0	19.5	18.5	18.5	16.5
	Coos Bay Station IV	2	94.5	20.0	19.5	19.0	18.0	18.0
	Yaquina Control	2	98.0	19.5	20.0	20.0	20.0	18.5
	Houston Channel Station I	2	80.0[a]	20.0	20.0	20.0	13.5[a]	6.5[a]
	Houston Channel Station II	2	85.0[a]	19.5	20.0	20.0	13.5[a]	12.0[a]
	Houston Channel Station III	2	58.5[a]	18.0	20.0	16.0[a]	0.5[a]	4.0[a]
	Houston Channel Station IV	2	75.0[a]	20.0	20.0	19.5	6.0[a]	9.5[a]
	Houston Channel Station V	2	77.0[a]	20.0	20.0	17.0[a]	7.0[a]	13.0
6	Yaquina Control	5	96.6	20.0	20.0	18.4	18.2	20.0
	Bailey Creek	5	81.2[a]	20.0	17.0[a]	17.6	12.2[a]	14.4[a]
7	Yaquina Control	5	91.8	20.0	20.0	17.2	18.4	16.2
	Skipanon River	5	90.0	20.0	19.4	19.6	16.6	14.4
8	Yaquina Control	5	93.8	20.0	19.8	16.8	18.8	18.4
	Raritan River	5	79.6[a]	20.0	19.2	17.0	9.4[a]	14.0[a]

[a] $P < 0.05$.

The Houston Channel Station III sediment was the most toxic of any tested in the bioassay experiments. Within the two replicates for that station, only nine of the initial 80 amphipods and cumaceans survived the 10-day exposure period, while 77 survived in the controls.

There was evidence of spatial differences in the toxicity of sediment along the Duwamish River-Pugent Sound transect (Experiment 3, Table IV). Lowest total survival was observed for sediment from the Turning Basin and Slip No. 1. The differences from the control were significant for both of these stations and for the Elliott Bay dump site. Mean total survival for Duwamish River mouth sediment was not significantly different from the controls. It was significantly different for the Puget Sound station, although none of the individual species' means differed significantly from the controls.

Mean survival of cumaceans was not significantly less than the control for any of the Duwamish River, Elliott Bay, or Puget Sound sediments (Experiment 3, Table IV). However, only between 2 and 13 ($x = 8.6$) cumaceans survived in the individual aquaria. The range of cumacean survival for the Yaquina Bay controls in all experiments was between 14 and 20 ($x = 17.5$). Because these ranges do not overlap, sediment along this transect is thought to be toxic to cumaceans, even though the means based on two replicates were not statistically different from the Experiment 3 control.

The sediment toxicity experiments revealed obvious interspecific differences in sensitivity. *Protothaca staminea* was not affected by any of the test sediments. The survival of both *Glycinde picta* and *Macoma inquinata* was significantly less than the control in only two instances, and crustaceans were always more sensitive than the polychaete or mollusks. The statistical significance of the difference between mean survival of *Paraphoxus epistomus* in the control and test replicates was the same as that of the entire assemblage in 16 of the 17 bioassays.

DISCUSSION

The solid phase of sediments from the Duwamish and Raritan rivers, Bailey Creek, and Houston Ship Channel retained sufficient toxicity to cause the acute mortality of benthic invertebrates. If such sediments were introduced in large quantities into the sea, the dump sites could become significant sources of marine pollution. The ultimate ecological

impact of polluted sediment disposal can only be determined through field or laboratory studies of chronically exposed biological communities. However, the type of short-term bioassay described here can provide data useful in the regulation of dumping activities.

The selection of appropriately sensitive benthic species is essential to the success of this bioassay in a regulatory program. The EPA/COE Implementation Manual [2] recommends that the five test species be selected from the natural dominants of benthic assemblages in the vicinity of the dump site. In agreement with the response of *Paraphoxus epistomus* in our experiments, the manual identifies infaunal amphipods as especially appropriate. The other test species must represent different taxonomic and trophic groups. If sublethal phenomena such as bioaccumulation are to be examined, it would be desirable to include species like *Protothaca staminea*, which can be expected to survive the exposure period.

Laboratory cultures exist for relatively few benthic species. Experience has shown that with reasonable care it is possible to collect test organisms from wild populations and maintain them under control conditions with low mortality. Because a variety of test species will be used in different coastal regions, the Implementation Manual suggests that a mysidacean shrimp of the genus *Mysidopsis* or *Neomysis* be included for interbioassay comparisons. These mysids can be maintained in continuous culture.[7, 8]

The test assemblage used in the experiments showed little, if any, sensitivity to burial under 15 mm of sediment or to alterations in grain size distribution. This is undoubtedly not true for all benthic organisms. The effects of these factors must be determined before other species are used in the bioassay.

The ocean dumping regulations [1] state that dredged material may be dumped only when the discharge will not exceed the "limiting permissible concentration" (LPC)—that is, a concentration that will not cause unreasonable toxicity. It is difficult to apply the LPC concept to the solid phase either in the water column or to sediment after it has settled to the bottom. The Implementation Manual [2] concludes that the results of the benthic bioassay provide an operational determination of the LPC. If mean survival in the test sediment is at least 10% less than the control mean and if the difference is significant at the 0.05 probability level, the LPC would by definition be exceeded. The percent differences between means which were statistically significant in our experiments ranged from 13.1 to 40.3% for the entire assemblage and from 15.0 to 97.5% for individual species.

The benthic bioassay has other applications in pollution control. Test sediment could be collected from wastewater and dredge material dump sites, ocean outfalls, or other benthic habitats suspected of being contaminated. The bioassay could indicate causal relationships between sediment toxicity and alterations in benthic community structure. Many regulatory programs emphasize water quality criteria, but less attention has been paid to sediment quality criteria.[9] Safe sediment concentrations for specific pollutants could be estimated through acute or chronic benthic bioassays. Chemical monitoring could then determine if the sediment criteria are exceeded.

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